*Environmental Health Perspectives*
*Vol. 99, pp. 233–235, 1993*

# Development of a $^{32}$P-Postlabeling Assay for 7-Methylguanines in Human DNA

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**The application of a $^{32}$P-postlabeling assay for 7-methylguanines in DNA was studied either by labeling the imidazole ring-opened dinucleotide derivatives or by using strong-anion-exchange column chromatography for the adduct enrichment from normal nucleotides. Data showed that 7-methylguanines can be efficiently labeled as dinucleotides when *in vitro* methylated DNA was first imidazole ring-opened and then digested to the dinucleotide level with deoxyribonuclease I, snake venom phosphodiesterase, and prostatic acid phosphatase. When using ion exchange chromatography for the adduct enrichment, DNA was digested with micrococcal nuclease and spleen phoshodiesterase. Anion exchange chromatography was applied for 7-methylguanine measurements in white blood cell DNA of healthy nonsmokers ($n$ = 17) and patients ($n$ = 4) treated with the methylating drugs procarbazine and dacarbazine. We found that the mean level of 7-methylguanine residues in nonsmokers was 2.5 per $10^7$ nucleotides. The corresponding level in the patient samples immediately after the drug treatment was 57 per $10^7$ nucleotides.**

## Introduction

The $^{32}$P-postlabeling assay has been used mainly in the analysis of large, nonpolar DNA adducts (*1,2*). There are few studies on the labeling of small alkyl adducts, particularly N7 guanine adducts (*1,3*). The problems relating to the stability of the N7 alkylation products can be solved by imidazole ring-opening in alkali (*4*). In the present study, a method to measure ring-opened 7-methylguanines as dinucleotides was developed. Anion exchange chromatography was also used for the isolation of 7-methylguanine nucleotides from normal nucleotides. By using this enrichment procedure, the levels of 7-methylguanines were determined in healthy individuals as well as in cancer patients receiving the methylating agents dacarbazine and procarbazine.

## Determination of 7-Methylguanines As Dinucleotides

2′-Deoxyguanylyl(3′-5′)-thymidine (dGpdT; P-L Biochemicals) and thymidylyl(3′-5′)-2′-deoxyguanosine (dTpdG; Sigma) were methylated at the N7 position of guanine by reacting with dimethyl sulfate (DMS; Fluka). The reaction products were purified by HPLC, lyophilized, and the imidazole ring of the 7-Methylguanine moiety was opened by alkali treatment. 7-Methylated dGpdT and dTpdG (Me-dGpdT, dTpdG-Me, respectively) and ring-opened 7-methylated dTpdG (dTpdG-ROM) were labeled quantitatively at femtomole levels. With ring-opened 7-methylated dGpdT (ROM-dGpdT), the limit of detection and the labeling efficiency were two orders of magnitude lower. Because of these result, we searched enzymes that would cut ROM-dGpdT but not dTpdG-ROM.

The efficiency of the enzymatic digestion was first tested separately with each of the enzymes DNAse I (Fluka), deoxyribonuclease II (DNase II; Sigma), micrococcal nuclease (Sigma), nuclease $P_1$ (Boehringer, Mannheim), nuclease $S_1$ (Sigma), prostatic acid phosphatase (Sigma), spleen phosphodiesterase (Boehringer, Mannheim) and snake venom phos-

**Table 1. Efficiency of the $T_4$ polynucleotide kinase reaction of ROM-dGpdT, dTpdG-Me, and dTpdG-ROM.**

| Adduct | Treatment | No. of experiments | % Recovery |
|---|---|---|---|
| ROM-dGpdT | ± DNA + enzymes | 1 | 0 |
| dTpdG-Me | ± DNA + enzymes | 1 | 0 |
| dTpdG-Me | DNA (1:4)[a] + NaOH + enzymes | 1 | 101 |
| dTpdG-Me | DNA (1:50)[a] + NaOH + enzymes | 1 | 83 |
| dTpdG-ROM | — | 2 | 91/100[b] |
| dTpdG-ROM | Enzymes | 2 | 31/56[b] |
| dTpdG-ROM | DNA (1:4)[a] + enzymes | 2 | 31/48[b] |
| dTpdG-ROM | DNA (1:50)[a] + enzymes | 2 | 33/56[b] |

Abbreviations: ROM, ring-opened methylated; Me, methylated; dGpdT, 2′-deoxyguanylyl(3′-5′)-thymidine; dTpdG, thymidylyl(3′-5′)-2′-deoxyguanosine.

[a]Me-guanine: DNA nucleotides.

[b]The results of two experiments.

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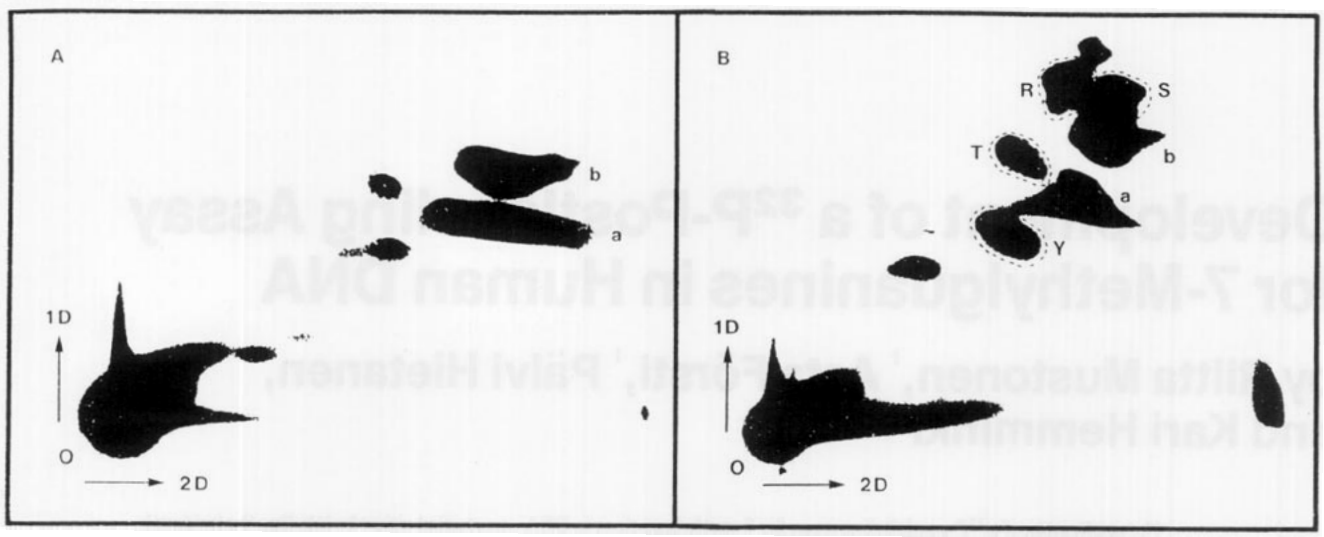


FIGURE 1. Two-dimensional resolution of $^{32}$P-labeled dinucleotide adducts from unmodified calf thymus DNA (*A*) and methylated DNA (*B*) after alkali treatment and enzymatic digestion. Y corresponds to ring-opened methylated 2′-deoxyguanylyl(3′-5′)-thymidine, and R, S, and T correspond to other alkali-treated methylated dinucleotides. O, origin containing radioactive ATP: a, orthophosphate; b, unidentified phosphorylation product formed in the incubation buffer containing the enzyme but no dinucleotide. 1D indicates the first dimension and 2D the second dimension of the TLC. The first dimension was developed in 0.2 M ammonium nitrate and 1% acetic acid and the second dimension in 0.5 M LiCl. Autoradiography was for 14 hr.

phodiesterase (Sigma). The only enzyme that digested dTpdG-Me and that did not digest dTpdG-ROM was snake venom phosphodiesterase. The mixture of enzymes DNAse I, snake venom phosphodiesterase, and prostatic acid phosphatase completely digested ROM-dGpdT and dTpdG-Me with and without added DNA, but it did not markedly digest dTpdG-ROM with or without added DNA (Table 1).

Preliminary experiments were carried out in the same manner to digest methylated and ring-opened methylated DNA (Me-DNA, ROM-DNA, respectively). Me-DNA was completely digested, but with ROM-DNA four adduct spots were found in the autoradiogram of a two-dimensional TLC (Fig. 1). The adducts co-migrated with the methylated, alkali-treated mixture of dTpdG, 2′-deoxycytidylyl(3′-5′)-2′-deoxyguanosine (dCpdG), 2′-deoxyadenylyl(3′-5′)-2′-deoxyguanosine (dApdG), and 2′-deoxyguanylyl(3′-5′)-2′-deoxyguanosine (dGpdG).

## 7-Methylguanine Levels in White Blood Cell DNA of Healthy Individuals and Cancer Patients Treated with Methylating Agents

Different prepurification methodologies have been applied for small alkyl DNA adducts (*2–6*). In present study, an anion exchange cartridge enrichment, which elutes the uncharged 7-alkylguanine residues in the void volume and retains the negatively charged normal nucleotides, was chosen for human samples (*5*).

Cancer patients, 24–77 years old, had undergone chemotherapy with dacarbazine or procarbazine for several days. The diseases were melanoma, lymphoma, and Hodgkin's diease. The blood was drawn at the end of the regimen. The cumulative doses at the end of the treatment varied between 1050 and 2800 mg (Table 2). All the patients were at least occasional smokers.

**Table 2. 7-Methylguanine levels in white blood cell DNA of cancer patients treated with methylating agents and in control individuals.**

| Patients | Treatment | Daily dose, mg | Total dose, mg | No. analyses/ individual | Adducts/$10^7$ nucleotides ± SD[b] |
|---|---|---|---|---|---|
| 1 | D | 300 | 1200 | 6 | 28 ± 11 |
| 2 | P | 150 | 1200 | 4 | 70 ± 35 |
| 3 | P | 200 | 2800 | 2 | 97 ± 43 |
| 5 | P | 150 | 1050 | 2 | 31 ± 1 |
| Total | | | | | 57 ± 33 |
| Controls | | | | | |
| 1–17 | — | — | — | 1 or 2 | 2.4(0.5–9.0)[c] |

[a]D, dacarbazine; P, procarbazine.
[b]The results were corrected by a factor of 2 for loss in preparation.
[c]Range in parentheses.

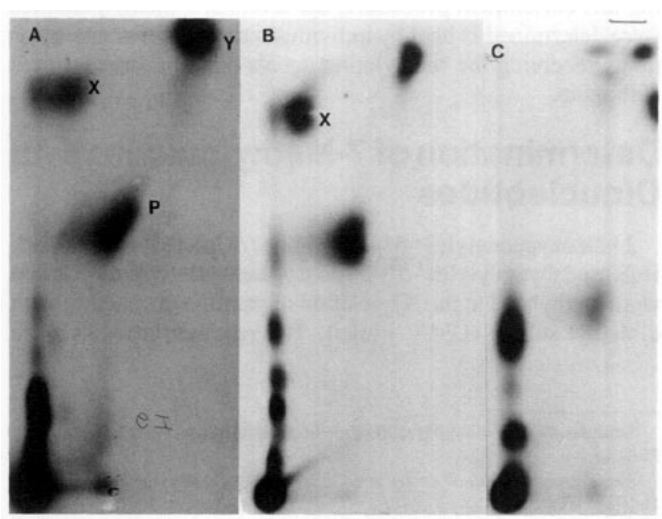


FIGURE 2. Two-dimensional separation of phosphorylated 7-methylated-dGMP standard (X) in panel (*A*); sample from a procarbazine-treated patient (*B*); and control person (*C*). P, orthophosphate.

The application of a two-dimensional TLC system to phosphorylated human samples is shown in Figure 2. The result showed a background level of 7-Me-dGMP in all the 17 normal healthy, nonsmoking individuals studied. However, the patients undergoing chemotherapy with methylating agents had some 20 times higher levels of 7-methylguanine in DNA than the healthy individuals (Table 2). Harris and co-workers reported 7-methylguanine levels in lung tissue at 10–50 adducts per $10^7$ guanines (*7*). Data for $O^6$-methylguanine from patients treated with procarbazine and dacarbazine gave adduct levels up to 18 and 28 methylguanines/$10^7$ nucleotides, respectively (*8,9*). This agrees with our data because the proportion of $O^6$/N7 methylguanine in rat DNA after a procarbazine dosage was shown to be about 0.1 (*10*). One has to be cautious, however, in the comparison of adducts with different repair kinetics.

This manuscript was presented as a poster at the Conference on Biomonitoring and Susceptibility Markers in Human Cancer: Applications in Molecular Epidemiology and Risk Assessment that was held in Kailua-Kona, Hawaii, 26 October–1 November 1991.

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